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REVIEW ARTICLE

Molecular systematics and population genetics of biological invasions: towards a better understanding of invasive species management

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Abstract

The study of population genetics of invasive species offers opportunities to investigate rapid evolutionary processes at work, and while the ecology of biological invasions has enjoyed extensive attention in the past, the recentness of molecular techniques makes their application in invasion ecology a fairly new approach. Despite this, molecular biology has already proved powerful in inferring aspects not only relevant to the evolutionary biologist but also to those concerned with invasive species management. Here, we review the different molecular markers routinely used in such studies and their application(s) in addressing different questions in invasion ecology. We then review the current literature on molecular genetic studies aimed at improving management and the understanding of invasive species by resolving of taxonomic issues, elucidating geographical sources of invaders, detecting hybridisation and introgression, tracking dispersal and spread and assessing the importance of genetic diversity in invasion success. Finally, we make some suggestions for future research efforts in molecular ecology of biological invasions.

Introduction

The recent extent of global trade and transport has led to an enormous displacement of biota across natural barriers into new environments, in some cases leading to the establishment of invasive populations. Species invasions are now considered a principal component in driving large-scale ecological changes given their ability to cause habitat degradation, lower native biodiversity (Wilcove *et al.*, 1998), contribute to ecosystem changes (D'Antonio & Vitousek, 1992; Vitousek, 1990) and change the evolutionary trajectories of species (Strauss *et al.*, 2006*a*). While it is difficult or in most cases impossible to put monetary values on impacts (Marais *et al.*, 2004), previous work has illustrated the devastating economic losses ascribed to invasive species of agricultural environments (Pimentel *et al.*, 2005). It is not surprising then that invasive species have been the research target in both natural and managed ecosystems to design appropriate management solutions, predict and prevent future invasions and restore previously invaded areas. Despite most of these research efforts being purely ecological, the field of invasion ecology still largely lacks reliable generalisations (Williamson, 1999), making the aforementioned research goals mostly of little use to land managers. This may reflect the tremendous amount of diversity involved in global biological invasions (taxonomic, environmental, reproductive, genetic, etc.), representing equally impressive variation in research findings aimed at identifying management, prevention and restoration solutions. For instance, whether a species' evolutionary ability or genetic variation plays an important role in invasion success remains speculative. Only recently have ecologists recognised that invasive species can evolve rapidly (for review, see Lee, 2002) and that such rapid genetically based adaptation might be more important in the success of biological invasions than was previously thought (Mooney & Cleland, 2001;

Sakai *et al.*, 2001). Alternatively, other studies question the importance of such variation and indicate phenotypic plasticity as key to colonisation success and the robustness of some species in recipient communities (e.g. Parker *et al.*, 2003; Poulin *et al.*, 2005; Le Roux *et al.*, 2007). This apparent paradox makes a generalisation about the role of genetic diversity in invasion success unattainable.

Molecular systematics and population genetics render new and exciting tools to better our understanding of population dynamics during biological invasions and have already shed light on management, prevention and restoration strategies. The use of molecular genetics, in particular, gained substantial interest in invasion evolution studies (Holland, 2000; Sakai *et al.*, 2001). Here, we review the current literature on molecular systematics and population genetics of invasive species and their application to the management and control of these species. First, we briefly introduce molecular systematics and population genetics and the types of molecular markers routinely employed in studying invasive species. Then, we review the application and usefulness of such data to management and control strategies and make suggestions for future research efforts in this emerging and exciting field.

Population genetics and molecular markers

Over time, the ultimate fate of a particular species in a particular environment will be determined by its biology and the demographic circumstances through which individuals are passed, for example reproductive success, migration, population size, natural selection and historical events. Population genetics focuses on describing the amounts and distribution of genetic variation within and among populations and how it pertains to demographic features. At the DNA level, genetic variation can arise through base substitutions (single nucleotide substitutions), insertion or deletion of DNA sequences (indels), inversion of DNA segments and the rearrangement of DNA segments. Over extensive evolutionary accumulation, many different instances of each type of mutation should be present in any given species, defining its genetic variation. The number of available molecular techniques and markers to study genetic variation has increased dramatically since the advent of DNA sequencing in the 1970s.

As a result of the differential actions of fundamental processes such as recombination, mutation and selective constraint, molecular markers differ in the amount of polymorphism that they display (Sunnucks, 2000). Marker choice is thus one of the most important aspects of any molecular genetic study (Baverstock & Moritz, 1996; Brower & DeSalle, 1994). The main concerns are whether the characters examined exhibit variation appropriate to the questions posed, have a clear genetic basis, and whether the data are gathered and analysed in such a way that one can compare and combine data derived from them (Moritz & Hillis, 1996). For example, a systematic study to detect cryptic species would normally use faster evolving markers that render high resolution on small temporal and time scales. On the other hand, if larger spatial and time scales were involved, slower evolving markers would be desirable. Using fast evolving markers for such large spatial scale studies will often lead to too much information, where the entities are too different with nothing linking them (Sunnucks, 2000). We briefly outline the most frequently used markers and their respective uses below. Table 1 also summarises the general characteristics of these markers.

Lastly, a plethora of statistical approaches, methodologies and computational software are available for analysis of population genetic data. These can vary widely in degrees of sophistication and we would advise new researchers to the field of molecular ecology to carefully consider all possible analysis types and their respective advantages and limitations for any particular data set(s). To review these approaches and computer software routinely used in population genetics here is beyond the scope of the current review and would be redundant. These subject areas have been extensively reviewed previously by others (Beaumont & Rannala, 2004; Beerli, 2006; Dudbridge, 2003; Felsenstein, 2003; Jones & Ardren, 2003; Knowles, 2004; Slatkin, 1995).

Allozymes – protein-based markers

Allozymes are nuclear-encoded enzymes that can be visualised by starch gel electrophoresis (Murphy *et al.*, 1996). DNA polymorphism may result in differences in the amino acid composition of polypeptide chains leading to different alleles that can be distinguished by their differential electrophoretic mobilities. These codominant Mendelian loci are routinely employed to determine standard estimates of genetic variation and gene flow (i.e. *F* statistics, Wright, 1978) within and among populations (Hamrick & Godt, 1990). Protein electrophoresis normally reveals only a small fraction of the genetic variation in a population and levels of polymorphism vary among different taxonomic groups (Parker *et al.*, 1998) but can be useful for situations where budget is limited and high-resolution data are not required. Other limitations include a lack of polymorphism for noncoding regions of the genome, nonneutrality and a tendency towards monomorphism despite underlying DNA sequence variation because of silent mutations.

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Table 1 Summary and characteristics of molecular markers routinely applied in molecular ecological studies of invasive species

Marker Type	Acronym	Variability[a]	Reproducibility	Precision[b]	PCR Assay	Single Locus	Inheritance	Allele Genealogy Feasible	Integrating Data Among Studies	Major Applications
Organellar										
Mitochondrial DNA	mtDNA	Low (multiple)	High	High	Yes	Yes	Maternal, codominant[c]	Yes	Direct	Maternal lineage, phylogeography, population genetic
Chloroplast DNA	cpDNA	Low (multiple)	High	High	Yes	Yes	Maternal, but sometimes paternal, codominant[c]	Yes	Direct	Parental lineage, phylogeography, population genetic
Multilocus nuclear										
Restriction fragment length polymorphism	RFLP	Low (2)	Intermediate	Intermediate	Few	Yes	Mendelian, codominant	No	Limited	Linkage mapping, genetic diversity
Randomly amplified polymorphic DNA	RAPD	Intermediate (2)	Low	Intermediate	Yes	No	Mendelian, dominant	No	Limited	DNA fingerprinting, population genetic, hybrid detection
Amplified fragment length polymorphism	AFLP	High (2)	High	Intermediate	Yes	No	Mendelian, dominant	No	Limited	Linkage mapping, population genetic
Intersimple sequence repeat	ISSR	High (multiple)	Intermediate	Intermediate	Yes	No	Mendelian, dominant	No	Limited	DNA fingerprinting, population genetic, hybrid detection
Single-locus nuclear										
Allozyme		Low (2–6)	Intermediate	Intermediate	No	Yes	Mendelian, codominant	Rarely	Direct	Linkage mapping, population genetic
Minisatellites		High (multiple)	High	Intermediate	Yes	Yes	Mendelian codominant	Rarely	Indirect[d]	Linkage mapping, population genetic, paternity analysis
Microsatellites	SSR	High (multiple)	High	Intermediate	Yes	Yes	Mendelian, codominant	Yes	Indirect[d]	Linkage mapping, population genetic, paternity analysis
DNA fragment sequence		Low/moderate	High	High	Yes	Yes	Mendelian codominant	Yes	Direct	Phylogeographic, population genetic

PCR, polymerase chain reaction.

[a]Number of alleles typically observed per locus are given in parentheses for each marker type.

[b]Differs from 'reproducibility' in that reproducible results may not accurately reflect the underlying variation, for example paralogous PCR products during RAPD analysis or the presence of null alleles in microsatellite analysis.

[c]mtDNA and cpDNA are haploid but represent one of numerous possible states, in contrast dominant markers the state is either present or absent.

[d]Data from these markers are indirectly, but meaningfully, integrated given adequate models of molecular evolution.

DNA-based markers

Broadly, DNA can be classified in two categories: nuclear DNA (nDNA) and organellar DNA [chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA)], both of which can be used to address different ecological questions. Nuclear genomes are much larger than organellar genomes, and depending on the ploidy level of the organism, normally represent multiple copies (genetic regions or loci) on homologous pairs of chromosomes. For example, a triploid organism would have three copies (alleles) of each region (locus). Nuclear DNA contains both unique single copy regions and repetitive (multiple copies) regions. Organellar genomes consist of coding segments such as ribosomal RNA genes or noncoding tandemly repeated units. Tandemly repeated units represent some of the most variable regions in the genome and are frequently used to construct molecular markers such as microsatellites and minisatellites. Organellar DNA is found in cytoplasmic structures and is inherited in a non-Mendelian fashion often with uniparental (usually maternal, but see Burban and Petit, 2003; Chat *et al.*, 1999) transmission.

Restriction fragment length polymorphism analysis

Restriction fragment length polymorphisms (RFLPs) reveal DNA polymorphisms as variations in restriction enzyme cleavage sites in DNA (Botstein *et al.*, 1980). Genetic variation because of mutational changes in restriction endonuclease recognition sites (4–8 bp) can be assessed by digesting DNA with specific restriction enzymes and gel electrophoretic visualisation. For nDNA, gene-specific probes are used in Southern blot hybridisation to visualise polymorphisms. However, RFLP variation can also be detected electrophoretically when dealing with smaller molecules such as mtDNA that normally yields fewer fragments (Tegelström, 1992). Another RFLP approach is to make use of the polymerase chain reaction (PCR) by digesting amplified DNA fragments with restriction endonucleases (Karl *et al.*, 1992).

Restriction fragment length polymorphism variation is generally low but in most cases, still sufficient to investigate genetic questions within and between populations. High costs of restriction endonucleases and intermediate to low reproducibility make RFLP analysis a less widely used approach.

Random amplified polymorphic DNA analysis

Random amplified polymorphic DNA (RAPD) markers produce banding patterns resulting from genome wide PCR amplification of uncharacterised fragments with short randomly chosen oligonucleotide sequences as primers (Williams *et al.*, 1990). RAPDs are presumed to be neutral and their variation detected as the absence or presence of PCR products as dictated by mutations at primer binding sites or indels in regions between primer binding sites. RAPD markers are inherited in a dominant Mendelian fashion, making differentiation between homozygous dominant and heterozygous individuals problematic. In addition, RAPDs are unable to distinguish between bands that are the result of distinct separate loci versus bands that are alleles from the same locus. For instance, two bands differing by substantial length because of an insertion or a deletion event between primer binding sites will in most cases be scored as two separate loci, alternatively, paralogous PCR products, that is different amplified sequences that are the same length, will be interpreted as a single locus. Reproducibility in RAPDs is normally low because of the low annealing temperatures used during PCR amplification. These markers have, nevertheless, successfully been used for species identification (e.g. Partis & Wells, 1996) and analysis of genetic variation within and among populations (e.g. Yue *et al.*, 2002). However, given the problems mentioned, RAPDs have become less preferable markers. Publication of data solely based on RAPD analysis has been denounced by many well-respected journals in population genetic and molecular ecological fields.

Amplified fragment length polymorphism analysis

Amplified fragment length polymorphism (AFLP) analysis is a multilocus PCR-based technique that combines the advantages and overcomes the limitations of both RFLP and RAPD analysis. As with RFLPs and RAPDs, the variation observed for AFLPs is generated by mutations in restriction enzyme cleavage sites and primer binding sites. AFLP markers are constructed by digesting total genomic DNA with restriction enzymes followed by the ligation of known adaptor sequences onto the ends of the resulting DNA fragments. These adaptors act as primer binding sites, enabling PCR amplification of fragments at high annealing temperatures. The tremendous number of fragments generated this way is reduced by the addition of known base pairs to the 3′ end of the adaptor-specific primers. These additional base pairs will extend past the fragment–adaptor ligated site and into the DNA fragment, only allowing annealing of the primer if the correct DNA sequence is present in the DNA fragment. This greatly decreases the number of PCR products to more manageable levels. Genetic variation is analysed in a similar fashion to RFLPs, but instead of analysing one locus at a time, many different loci can be analysed simultaneously. As with RFLPs, the dominant inheritance of AFLPs reduces their informative

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ability, but the large numbers of polymorphisms revealed (often exceeding 100) and high reproducibility because of higher annealing temperatures make them more efficient markers for inter- and intrapopulation studies.

Variable number of tandem repeats

Variable number of tandem repeats (VNTRs) are generally noncoding and thus selectively neutral (Li *et al.*, 2002) genomic regions that consist of several to multiple copies of the same DNA sequence repeated tandemly. These repeat units can vary in length from 2 to 64 bp. When repeats are 2–9 bp in length, the simple sequence repeats (SSRs) are referred to as microsatellites, for example $[CA]_6$ or [CACACACACACA]. Microsatellite sequences have been found in all organisms studied to date and tend to be evenly distributed in the genome on all chromosomes. These regions are hypervariable and mutation rates of 10^{-2} per generation have been reported (Weber & Wong, 1993). Polymorphisms are generated by single-strand slippage during DNA replication resulting in differences in the number of repeat units (Schlotterer & Tautz, 1992). Given the high rate of mutation, it is not uncommon to find a large number of alleles per locus (up to 20–50 alleles). Microsatellite sequences are normally flanked by more conserved DNA regions enabling amplification through locus-specific primers. Primers can be labelled with fluorescent dyes, enabling high-throughput genotyping on automated sequencers. This, coupled with the high levels of polymorphism and codominant Mendelian inheritance, makes microsatellites some of the most informative markers currently available. This is clearly reflected in the number of studies that employ them to determine genetic diversity at the population level, genome mapping, parentage and kinships. Microsatellites are unfortunately time consuming and expensive to develop.

Longer repeat units (>10 bp) are called minisatellites and as with SSRs are generally noncoding, neutral markers and distributed throughout the genome. Minisatellite polymorphisms can be detected using a variation of the RFLP method. Following restriction digestion of whole genomic DNA and fragment separation, Southern blot analysis is conducted with a VNTR-specific probe. Typically, one probe detects VNTRs at many different variable loci throughout the genome, giving rise to a multibanded genotypic fingerprint. Because of their high variability, minisatellites are frequently employed for individual identification, forensics and paternity exclusion (Bachmann, 1994).

Intersimple sequence repeats (ISSR) analysis combines the advantages of high polymorphism levels exhibited by microsatellite sequences and the relative ease of applying RAPD- or AFLP-based PCR amplification. A multilocus fingerprint is generated by amplification of fragments flanked by oppositely oriented microsatellites using microsatellite core sequences and a few selected nucleotides as a single primer. The high variability of these regions (primer binding and PCR fragment length) can reveal minute levels of genetic variation (Wolfe & Liston, 1998). Dominant ISSR loci generally have higher levels of polymorphism than RAPDs and higher annealing temperatures allow for greater reproducibility. ISSRs can be used to infer relationships among closely related taxa (Wolfe *et al.*, 1998).

Gene sequencing

Point mutations are the most abundant polymorphisms and are often missed by other molecular methods such that gene sequencing provides the ultimate level of resolution of genetic variation. Various universal and taxon-specific primers are available, enabling researchers to amplify precise gene sequences of known function for analysis. Primer binding sites usually entail highly conserved regions that flank variable gene sequences exhibiting polymorphisms. Because substitution rates of different loci vary widely, selecting appropriate markers can be guided according to the intended ecological application and the taxonomic level at which the questions are focused. For example, for phylogenetic reconstruction between metazoan genera, slower evolving nuclear genes such as elongation factor 1 alpha might more accurately reflect evolutionary relationships among taxa than a faster evolving gene such as the mitochondrial gene cytochrome oxidase *I*. Longer divergence times associated with taxa in deeper parts of the phylogeny will lead to homoplasy, where characters are identical by state but not because of descent, also termed saturation. When using gene sequences that evolve too fast, phylogenetic signal is lost and erroneous phylogenetic relationships can be inferred. For plants, however, the mithocondrial genome often evolves at very slow rates, even slower than nuclear genomes (Wolfe *et al.*, 1987, but see Cho *et al.*, 2004), while plastid genomes normally evolve at rates higher than mtDNA and nDNA.

Genealogies constructed from DNA sequence data and their relationship to demographics are a major area of expansion, revealing previously unimaginable advances in what can be deduced about population processes and history. For example, molecular approaches can help distinguish current demographic processes from the effects of historical events (Templeton, 1998).

The differential patterns of evolution and transmission in nDNA versus organellar DNA cause genealogies derived from them to reflect different aspects of population

biology and history (Rubinoff & Holland, 2005). Combining such data is useful in unravelling population processes that would otherwise be extremely challenging. Examples of these include detecting hybrid individuals, sex-biased dispersal and asymmetrical mating preferences.

Application of molecular genetics to invasive species management

Invasive species are notoriously difficult and expensive to control and usually impossible to eradicate. Thus, it is of utmost importance to identify the most efficient management strategies available (Byers *et al.*, 2002). Prevention is clearly more cost-effective than post-establishment eradication or containment, but in most cases, alien species are only identified after successful establishment. Here, we focus on the application of molecular markers to the management of invasive species by resolution of taxonomic issues such as the precise, repeatable identification of introduced species and cryptic species, elucidation of geographic source(s), detection of introgression, tracking dispersal and spread and, last, the role of genetic diversity in invasive success.

Taxonomic identity

Identifying introduced species and their point of origin are often problematic for various reasons, including high species diversity, poor or outdated taxonomy in source regions, introduction of multiple sympatric, cryptic taxa and multiple introductions (Stepien & Tumeo, 2006). Taxonomic misidentification may obscure accurate invasion history and preclude appropriate management strategies, for example population regulation below economic threshold or containment to a particular area.

Although integrated approaches to manage invasive species can be useful across multiple taxa, correct taxomonic identification can aid in determining the most effective management strategies. Some management practices, in particular, biological control, may be more productive or only effective against certain species or even variants (genotypes or biotypes) within a species (e.g. Goolsby *et al.*, 2006). Such inter- and intraspecific variants are often impossible to distinguish based on morphology alone, whereas molecular markers can often reliably, rapidly and accurately identify variants and cryptic taxa.

Molecular systematics approaches are particularly useful in invasions of aquatic environments where factors limiting dispersal and reproduction are not well understood (Palumbi, 1997). A comprehensive report by Booth *et al.* (2007) gives an excellent overview of how the use of molecular systematics helped in elucidating taxonomic issues in various morphologically conservative invasive seaweed species. For example, using an internal transcribed spacer (*ITS*) phylogenetic analysis, Jousson *et al.* (2000) showed that the seaweed *Caulerpa taxifolia* invasive in the Mediterranean basin is closely related to strains cultivated in European aquaria. Booth *et al.* (2007) furthermore discuss the role of molecular approaches that have been applied to seaweed invasions that are also relevant to various other sections throughout this manuscript, for example identification of source regions, dispersal and hybridisation (see other subsections under this section). Similarly, Holland *et al.* (2004) showed that morphologically uniform introduced populations of upside-down jellyfishes, *Cassiopea* spp., in Hawaiian coastal water represent two genetically distinct lineages. Furthermore, Holland *et al.* (2004) showed that these two cryptic species resulted from separate introductions from different geographic sources and therefore likely arrived by separate vectors. Another study that involves discrimination between invasive and noninvasive species of oyster drills comes from Garcia-Meunier *et al.* (2002). Monomorphism between egg capsules and juveniles of introduced Japanese oyster drills, *Ocinebrellus inornatus*, and native European oyster drills, *Ocenebra erinacea*, compromised ongoing research efforts to assess predation risks on commercially cultivated oysters and mussels and environmental impacts of introduced drills (Garcia-Meunier *et al.*, 2002). Characterisation of unique nuclear sequences from each species provided baseline information for fast and reliable identification of any life stages for both species (Garcia-Meunier *et al.*, 2002). These markers can be employed to determine ecological impacts (abundance and distribution) of *O. inornatus* and its influence on native *O. erinacea*. An interesting example of the consequences of taxonomic misidentification ironically involves some of the best studied aquatic invaders in North America, dreissenid mussels. Until 1992, it was assumed that all US invasions involved a single species of zebra mussel, *Dreissena polymorpha*. Genetic analysis using allozyme polymorphisms revealed the presence of a second species, *Dreissena bugensis* (quagga mussel) (May & Marsden, 1992) that is currently displacing zebra mussels in parts of the Great Lakes (Jarvis *et al.*, 2000). This taxonomical error puts the validity of previous life history experiments, specific invasion protocols and even genetic inferences about founder effects in doubt. This example illustrates accurate taxonomic identification as a fundamental first step to biological characterisation of introduction events (Holland, 2000). Native predators of dreissenid mussels, round gobies, *Neogobius melanostomus*, and tubenose gobies, *Proterorhinus marmoratus*, were also accidentally introduced into

the Great Lakes and are now dominant benthic species (Jude, 2001). These species belong to the species rich but poorly understood subfamily, Neogobiinae, and occur sympatric with numerous neogobiin species in their native ranges (Stepien and Tumeo, 2006). Neogobiine adaptability to heterogeneous habitats makes them good invaders and, coupled with the group's taxonomic uncertainty, prompted Stepien & Tumeo (2006) to develop DNA markers to evaluate whether additional cryptic species are present in the Great Lakes. Part of the mtDNA genome proved diagnostic for all species at all life stages. No additional cryptic species were detected in the Great Lakes, but it is predicted that neogobiin congeners will invade in the future from the Ponto-Caspian region, making these markers valuable for rapid and early detection of future introduction events.

Among terrestrial organisms, plants are particularly notorious for having taxonomic uncertainties because of interbreeding in and among species complexes, introgression among closely related species and high levels of phenotypic plasticity. Le Roux *et al.* (2006) used internal transcribed spacer (*ITS1* and *ITS2*) sequence data to resolve species of invasive fireweed in the *Senecio madagascariensis* complex. This complex encompasses morphologically similar species that are frequently mistaken for one another. In their phylogenetic reconstruction, Le Roux *et al.* (2006) resolved a potential native origin for Hawaiian fireweed infestations. These results will have important implications towards selecting host-specific biological control agents. Another good example of a study that used molecular markers to resolve species boundaries in a taxonomically difficult plant group focused on pigweeds, *Amaranthus* spp. (Wetzel *et al.*, 1999). Pigweed species are aggressive competitors and produce allelopathic chemicals making them particularly noxious weeds in agricultural areas. In their study, Wetzel *et al.* (1999) developed diagnostic markers for native and exotic *Amaranthus* species, allowing fast and reliable identification. Similarly, Saltonstall (2002) used mtDNA markers to show that invasive populations of North American *Phragmites australis* represent a unique invasive haplotypic variant that differs from native populations. Furthermore, Burdick & Konisky (2003) correlated landscape disturbance levels and enhanced aggressiveness of this invasive haplotype, a finding that can be incorporated in management protocols: management prioritisation of disturbed habitats and the reduction of human disturbances in vulnerable areas. Such decisions are based on a case-by-case basis and incorporate rapid discrimination between the invasive and the native haplotypes using diagnostic RFLP markers (Saltonstall, 2003).

The studies cited above illustrate both the importance of sound taxonomy and the efficacy of molecular techniques towards this end when dealing with invasive organisms. In each example, taxonomic identification was the first step towards making effective management decisions.

Determining the native provenance of invasive species

Invasive species often have large native ranges (Lodge, 1993) implying that it is not always easy to determine their geographical sources. It is of utmost importance to identify geographical origins when considering biological control as a management option. Biological control is the most economic and self-sustained control strategy in natural ecosystems (Messing & Wright, 2006) with about one in three attempts resulting in enemy establishment (Hall & Ehler, 1979) and half of these leading to complete control of the target species (Hall *et al.*, 1980). A survey of successful biological programmes showed that most effective natural enemies are usually host specific (Rosen, 1986). Failure to correctly identify invasive species' native origins and geographical sources could lead to unsuccessful enemy establishment, poor host specificity and/or incomplete control. This is especially true when dealing with biotypes of a single invasive species (e.g. Chaboudez, 1994), invasive species complexes [e.g. *S. madagascariensis* (Le Roux *et al.*, 2006)] or natural enemy host races [e.g. skeleton weed rust, *Puccinia chondrillina* (Espiau *et al.*, 1998)].

An example of determining the native provenance of an alien species and its direct application to biological control comes from invasive *P. australis* discussed earlier (Saltonstall, 2002). Determining the native origin of the invasive haplotype in Europe led to the discovery of specialised herbivore complexes and was a considerable advancement for the future control of this species (Häfliger *et al.*, 2006).

In a similar study, Scott *et al.* (1998) showed that invasive populations of *Chromolaena odorata* in Australia encompass early- and late-seasonal flowering types, corresponding to two distinct *ITS1* genotypes. Coincidently, these two genotypes were also found occurring sympatrically only in Brazil, revealing Brazil as the native source of Australian infestations. Gwiazdowski *et al.* (2006) did a phylogeographical study in an attempt to identify regions to explore for co-evolved natural enemies of the North American invasive beech scale, *Cryptococcus fagisuga*. A mtDNA-based phylogeny suggested that the subspecies *Fagus sylvatica* spp. *orientalis* is the native host of *C. fagisuga* and that natural enemies would be best sought on oriental beech in northeastern Greece, the Black Sea drainage basin, the Caucasus Mountains and northern Iran. In another example, Milne & Abbott (2004) used cpDNA RFLPs and RAPDs to elucidate the

most likely geographical source(s) of Privet, *Ligustrum robustum*, in the Mascarene Islands. Their analyses concluded that cpDNA haplotypes were identical for introduced and native Sri Lankan subspecies, *L. robustum* spp. *walkeri*, differing from other native range regions.

Unfortunately, to date, very few studies have simultaneously investigated the native provenances of invasive species and the productivity of biological control agents from source regions. Typically, molecular biologists share phylogeographical data with authorities responsible for biological control programs. A recent study by Goolsby *et al.* (2006), however, gives an excellent example of such integration. Goolsby *et al.* (2006) analysed cpDNA sequence data of the Old World climbing fern (*Lygodium microphyllum*), an invasive species in Florida, USA. The invasive haplotype was compared with those from the native range of *L. microphyllum* and subsequently led to an identical haplotypic match. *Floracarus perrepae*, the natural enemy of this fern throughout its native range, was also collected from all native range populations included in this study. Not surprisingly, the *F. perrepae* genotypes collected from the identified source population proved to be the most productive and damaging to *L. microphyllum* populations in Florida.

Determining the geographic source(s) of invaders is important not only to the success of biological control programs but also to understand basic processes in invasion ecology. Molecular techniques provide the only reliable way to determine source region(s) for species with large native ranges where introduction and invasion histories are not well documented.

Hybridisation, introgression and invasiveness

Genetic recombination as a result of hybridisation and introgression will almost certainly have ecological consequences and it has been suggested that this might be a strong determinant of high fitness in invasive species (Ellstrand & Schierenbeck, 2000). Hybridisation, gene flow and admixture will infuse genetic diversity and novel genotypes, masking deleterious alleles and transferring favourable ones (Abbott, 1992). In their review, Rieseberg & Brunsfeld (1992) showed that morphological evidence of introgression might be misleading or not evident at all. The contribution of molecular methods to detect and understand introgression and hybridisation is already substantial.

Ellstrand & Schierenbeck's (2000) excellent review gives convincing evidence for numerous plant species where hybridisation preceded the emergence of invasive populations. A treatment of this report here would be redundant and we only mention some of the examples cited in their review to focus on more recent reports.

A textbook example of hybridisation leading to the evolution of an invasive species concerns cordgrass, *Spartina*. The highly aggressive hybrid *Spartina anglica* originated in England as a result of hybridisation between native cordgrass *Spartina maritima* and introduced *Spartina alterniflora* (Ferris *et al.*, 1997). *S. alterniflora* was also introduced on the west coast of the USA and using RAPD markers, Daehler & Strong (1997) showed that hybridisation occurred with another native cordgrass species there, *Spartina foliosa*. These hybrids display vigorous growth as a result of greater pollen and seed output (Ayres *et al.*, 2004) and are threatening to displace native *S. foliosa* populations. Another salt marsh plant, *Sarcocornia perennis*, is an example where hybridisation led to novel mechanisms of successional invasion and species replacement (Figueroa *et al.*, 2003). RAPD markers confirmed that the invasive genotype of *S. perennis* is a hybrid between *S. perennis* and *Spartina fruticosa*. This hybrid is now becoming the dominant species in salt marsh environments where it displaces its progenitors. Similarly, Bleeker (2003) used cpDNA markers and AFLP markers to show that hybridisation between two Brassicaceae species, *Rorippa austriaca* (invasive) and *Rorippa sylvestris* (native), led to the evolution of a new invasive taxon, *Rorippa* × *armoracioides*, in Germany. Furthermore, the molecular data suggested that these two species are likely to hybridize wherever they are sympatric. These results have direct management applications. In addition to containing *R. austriaca* populations, potential contact zones between *R. austriaca* and *R. sylvestris* should be prioritised for eradication efforts of *R. austriaca*. Abbot & Forbes (2002) resolved the origin of invasive *Senecio cambrensis* in the British Isles as a hybrid between the native *Senecio vulgaris* and an introduced species *Senecio squalidus*. The relative ease of hybridisation between these two species was illustrated by identifying multiple origins of hybrids (Abbot & Forbes, 2002). Milne & Abbot (2000) used a combination of chloroplast and nuclear RFLPs to confirm that *Rhododendron ponticum* reached England from the Iberia Peninsula and that hybridisation occurred in its northern British range with yet another exotic *Rhododendron* species, *Rhododendron catawbiense*. Hybrid populations acquired increased tolerance to low temperatures leading to higher fitness and more aggressive invasive behaviour (Milne & Abbot, 2000). Similarly, in the USA, it was found that highly invasive *Tamarix* species from Eurasia were hybridising in their invasive range (Gaskin & Schaal, 2002). The most devastating and abundant *Tamarix* genotype proved to be a hybrid between two previously introduced species. Pyšek *et al.* (2003) reported that introduced *Reynoutria* taxa and their associated hybrids show differential vegetative regeneration rates. Hybrids regenerated better than their

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closely related parents, a crucial advantage to a species that spread by water. Hybrid taxa reproduce mostly vegetatively that renders reproductive assurance and fixes new hybrid combinations, contributing to their abundance and persistence.

Increased invasiveness as a result of hybridisation has also been documented for aquatic species. Moody & Les (2002) investigated the relationship between invasive and native watermilfoil (*Myriophyllum* spp.) in North America. DNA sequence data obtained from the nuclear (*ITS*) genome for four different *Myriophyllum* spp. (*Myriophyllum heterophyllum, Myriophyllum pinnatum, Myriophyllum spicatum* and *Myriophyllum sibiricum*) indicated that extremely invasive populations (monospecific stands) were always characteristic of hybrid populations with parental populations lacking aggressive growth. The aquatic weevil, *Euhrychiopsis lecontei*, is a host-specific biological control against exotic *M. spicatum* with no significant impact on native *M. sibiricum*. However, recently, some *M. spicatum* populations showed resistance against *E. lecontei*. Moody & Les (2002) confirmed that these were hybrid populations that acquired resistance from the native *Myriophyllum sibricum* parental lines. These findings will have serious implications for the current and future biological control of *Myriophyllum* spp. The marine alga, *Caulerpa racemosa*, was thought to have reached the Mediterranean Sea in the mid-1920s from the Red Sea (Durand *et al.*, 2002). Stationary populations of *C. racemosa* were distributed throughout the Mediterranean Sea until the early 1990s after which some populations started spreading rapidly. Morphological characterisation led to the recognition of three distinct taxa within the *C. racemosa* complex, one of which represents the invasive taxon (Verlaque *et al.*, 2000). Phylogenetic reconstruction based on *ITS* and *18S* rDNA sequence data identified a recent hybridisation event between two varieties of *C. racemosa* and as a result of heterosis led to invasive hybrid populations (Durand *et al.*, 2002).

While the correlation between hybridisation and invasive success has been extensively documented for plants, similar treatments for other organisms are lagging. Facon *et al.* (2005) showed that hybridisation led to superior competitiveness of two morphs of the invasive freshwater snail, *Melanoides tuberculata*, over their parental lineages. They also showed that fecundity was lower in hybrid lines but that, as a result of heterosis, a shift in life histories towards larger investment in juvenile biomass and growth has occurred. Invasion by rusty crayfish, *Orconectes rusticus*, in the USA has led to the rapid displacement of native species. Perry *et al.* (2002) used diagnostic nDNA markers to detect hybridisation between *O. rusticus* and *Orconectes propinquus* and inferred that hybrids are displacing native *O. propinquus* populations. Interestingly, displacement of *O. propinquus* was not because of hybrid vigour but was hypothesised to be the result of biased mating patterns (*O. rusticus* males outcompeting *O. propinquus* males). Unfit hybrid progeny decreases the reproductive output of *O. propinquus* or alternatively intermediate hybrid progeny outcompetes *O. propinquus* males for mating with *O. propinquus* females.

Hybridisation preceding invasiveness is particularly relevant to genetically modified (GM) crops and their close wild relatives. Evidence for conventional gene flow (not involving transgenes) and hybridisation between cultivated and wild populations are numerous (e.g. *Sorghum*, Arriola & Ellstrand, 1996 and *Brassica*, Rieger *et al.*, 2002). Genetic modification of crops is now commonplace and normally involves the insertion of genetic material to express resistance against the effects of herbivores and parasites (e.g. *Bt* corn) or herbicides (e.g. Roundup ready soybean). Introgression of transgenes into wild relatives may render the same resistance mechanisms to hybrids, leading to novel and enhanced fitness traits and increased persistence in agricultural and natural ecosystems.

Hybridisation between GM crops and their close relatives remains largely untested. Warwick *et al.* (2003) used AFLP markers to show that wild *Brassica rapa* populations acquired glyphosate resistance through hybridisation with commercial genetically modified canola in Canada. Canada is also currently commercially producing canola engineered for glufosinate- and bromoxynil resistance implying that these traits also have potential to escape into wild populations of close relatives. Furthermore, Halfhill *et al.* (2004) showed that transgenic hybrids of *B. rapa* have the potential to produce viable transgenic seeds in backcrosses. Glyphosate transgene escape into native creeping bentgrass, *Agrostis stolonifera*, populations in the USA was recently reported by Reichman *et al.* (2006). Nuclear and cpDNA-based gene phylogenies revealed that both seed dispersal and pollen drift from GM crops into wild populations were responsible for transgene escape. Transgenes would persist in wild *A. stolonifera* if constant selection pressure (herbicide application) ensures fitness advantages to hybrids, leading to weedy populations in agricultural ecosystems.

Molecular techniques remain the most reliable and conclusive approach to detect transgene escape through introgression. These studies will become more commonplace as more evidence accumulates on hybridisation between GM and non-GM plants and would help in resolving management strategies and regulations. The positive correlation between hybridisation and increased invasiveness illustrates how the detection of such events will improve management strategies, for example prioritisation of contact zones for eradication, isolation distances between GM

crops and their wild relatives, removing or reducing pressures such herbicide application rates that select for introgressed transgenes.

Dispersal and spread of invasive species

Dispersal is a critical factor for the success and self-sustainability of introduced species (Shigesada & Kawasaki, 1997). Quantifying dispersal, especially rare long-distance dispersal, is notoriously difficult and time consuming when measured directly. This problem is exacerbated in plant populations, especially for past dispersal events. Highly variable molecular markers now render the ability to quantify the movement and spatial distribution of alleles (gene flow), indirectly measuring dispersal as a function of individual, seed and pollen movement (Ouborg *et al.*, 1999). First, the distribution of alleles among populations is determined, a population genetic model applied and the amount of gene flow that would result in a similar distribution inferred. Second, an indirect inference of dispersal pattern(s) is carried out by spatial regression of geographical distance and genetic distance or spatial autocorrelation analysis. Alternatively, individual-based assignment tests that assign individuals probabilistically to candidate populations by their genotypic makeup can be employed.

The efficiency of this indirect approach was illustrated in a study by Berry *et al.* (2004) that demonstrated dispersal patterns of grand skink, *Oligosoma grande*, as inferred from microsatellite markers and individual-based assignment tests corroborated patterns previously inferred from a 7-year long mark-and-recapture study of the same populations. This example clearly shows the reduction in time and costs when employing such alternative methods. A better understanding of invasive spread and dispersal patterns has important implications for management. For instance, eradication of small pioneering populations in front of a continuous invasion front can be the most effective means of slowing or even stopping spread (Moody & Mack, 1988). Alternatively, invaders exhibiting long-distance expansion may need biological control agents capable of dispersing over equally long distances to keep up with their targets (Fagan *et al.*, 2002). Some species are capable of recolonising areas from which they were previously eradicated, emphasising the need for simultaneous eradication efforts of connected populations (Hampton *et al.*, 2004).

Recently, Hampton *et al.* (2004) used microsatellite markers to infer dispersal patterns of feral pigs (*Sus scrofa*) invading parts of Western Australia. Because of public health threats posed by feral pigs near water reservoirs control programs specifically targeted these areas, but re-invasion hampered eradication efforts. Genetic structure indicated that dispersal during re-invasion occurs almost exclusively from upper reaches within the same watercourse/river system and not from nearby neighbouring systems. Furthermore, the study identified unidirectional 'source' populations for subpopulations that were subjected to eradication efforts. More effective eradication will thus be achieved by simultaneously targeting source populations and populations within the same catchments (Hampton *et al.*, 2004). In another example, the dispersal patterns of the brown rat, *Rattus norvegicus*, were indirectly determined using individual-based assignment tests, on South Georgia Island (Robertson & Gemmell, 2004). The current extent of brown rat invasions makes eradication in a single effort economically unfeasible and potentially unsuccessful because of recolonisation by survivors. However, Robertson & Gemmell's (2004) study indicated that extensive glaciation subdivides the island's rat populations. Glaciers act as barriers to dispersal, leading to units of manageable size for eradication efforts without recolonisation risk from neighbouring populations. Viard *et al.* (2006) investigated the dispersal capabilities of the introduced slipper limpet, *Crepidula fornicata*. Spatial modelling using population genetic differentiation and a two-dimensional hydrodynamic model confirmed the same patterns of dispersal, namely (a) high-density populations capable of releasing large numbers of larvae to neighbouring populations and (b) that larvae can disperse over very long distances within short-time periods (21 days). Evidently, the management policy for *C. fornicata* invasion needs to start from the very beginning of its introduction, including larvae as part of the management scheme. Keller (2000) showed a strong correlation between the distribution of genetic variation in *P. australis* and the geographic distance alongside rivers, indicative of downstream dispersal. More recently, Walker *et al.* (2003) used microsatellite data and found the same general pattern for *Heracleum mategazzianum* introduced to England. However, for this species, several independent secondary introductions by human-mediated transport were also detected (Walker *et al.*, 2003). Until recently, the invasive spread of *D. bugensis* (quagga mussel) in the Laurentian Great Lakes was thought to resemble a gradual diffusion from the initial introduction in Lake Erie (Mills *et al.*, 1993). Microsatellite markers showed that leptokurtic dispersal (combination of rare long-distance dispersal with diffusive settlement) was the predominant pattern of dispersal (Wilson *et al.*, 1999). Such data can be applied to illuminate the predicted ranges of quagga mussel invasions (Wilson *et al.*, 1999). A similar approach was applied to endemic and invasive quagga mussels in Eurasia (Therriault *et al.*, 2005). In contrast to Wilson *et al.* (1999), these Eurasian populations failed to support

isolation by distance, indicating that long-distance dispersal through boats frequently occurs within these regions (Therriault *et al.*, 2005). Recently, Williams *et al.* (2007) investigated the spatial genetic structure of the invasive Brazilian peppertree, *Schinus terebinthifolius*, in Florida, USA. Spatial structure of cpDNA haplotypes and nuclear microsatellite loci were applied to a geostatistical model to estimate dispersal. The results indicated a directional genetic cline and evidence for short-distance genetic spatial autocorrelation as well as occasional long-distance jumps exist (stratified dispersal). It was speculated that areas previously subjected to eradication efforts are vulnerable to rapid recolonisation, suggesting the need for concerted eradication efforts over large areas or the introduction of an effective biological control agent against *S. terebinthifolius*.

The indirect approach to infer dispersal from molecular data is a relatively novel approach and we anticipate that more studies will pursue this time and cost-effective avenue. The application of geographic information systems data to such studies would prove particularly useful to further correlate dispersal with environmental factors (Prather & Callihan, 1993).

Genetic diversity

Population genetic theory predicts that high genetic diversity predisposes invasive populations to success at establishing, persisting and dispersing into novel habitats. It is not surprising that more population geneticists are turning their attention towards invasive species as model systems. The inference of the amount and distribution of genetic variation could also contribute towards better management practices.

Evolution in recipient communities that resulted in increased fitness and invasive success has been documented for numerous species (e.g. Bone & Farres, 2001; Maron *et al.*, 2004). In most cases, especially intentional horticultural or agronomical introductions, elevated levels of genetic diversity resulted from multiple introduction events. The strong correlation between higher genetic diversities and multiple introductions has been extensively documented for many different invasive species, for example *Ambrosia artemisiifolia* introduced to France (Genton *et al.*, 2005) and *Cirsium arvense* introduced to USA (Slotta *et al.*, 2006). Consistent with this interpretation, Sexton *et al.* (2002) found evidence for genetic variation in root biomass and subsequent local adaptation in invasive populations of salt cedars, *Tamarix ramosissima*, in the USA. These invasive populations arose from multiple introductions from different native range regions and accumulated genetic diversity that facilitated local adaptation. Selection for adaptive genetic variation has been shown for a number of introduced species, for example *Heracleum* species (Umbelliferae) (Jahodová *et al.*, 2007), *Conyza* species (Asteraceae) (Thebaut & Abbott, 1995) and *Solidago* species (Asteraceae) (Weber & Schmid, 1998). More recently, Lavergne & Molofsky (2007) provided evidence that repeated introductions of reed canarygrass, *Phalaris arundinacea* L., to the USA resulted in exceptionally high levels of genetic diversity. Subsequent recombination of this native, continental-scale genetic diversity, led to the establishment of novel, highly invasive genotypes.

Alternatively, genetically diverse source populations could harbour variants that are 'pre-adapted' to conditions in the recipient environment. In these instances, it is just a matter of introducing the right genotype(s) into the right environment(s). An example of a pre-adapted genotype leading to invasive spread comes from Neuffer & Hurka (1999). Invasive populations of *Capsella bursa-pastoris* showed quantitative ecotypic variation similar to that observed in native ranges (Neuffer & Hurka, 1999). Here, multiple introductions led to the successful establishment and spread of pre-adapted genotypes.

From a management perspective, the abovementioned studies caution against repeated introductions of exotic species. This may be particularly applicable to agronomically or horticulturally important species that have been inbred for various traits and for species with mixed breeding systems, as sexual reproduction facilitates recombination and asexual clonal reproduction preserves successful genotype(s). Intentional introductions should, as a precautionary mechanism, focus on importing founders containing low levels of genetic variation.

Additionally, genetic diversity may help predict the potential of invasive populations to evolve resistance to management practices such as herbicide or biological control. Invasive cordgrass, *S. alterniflora*, harbours genetic variation in both tolerance and resistance to its introduced biological control agent, *Prokelisia* hoppers (Garcia-Rossi *et al.*, 2003). Similarly, Hufbauer & Via (1999) found that the pea aphid, *Acyrthosiphon pisum*, shows genetic variation in resistance to parasitism by its parasitoid *Aphidius ervi*. Peever *et al.* (2000) found that the chestnut blight fungus, *Cryphonectria parasitica*, exhibits genetic variation in tolerance to strains of its hypovirus control agent. These examples illustrate that tolerance or resistance to control practices can be achieved in populations containing high levels of genetic diversity. Detection of high levels of genetic diversity in invasive populations [potentially equal or higher than in native populations (e.g. Lavergne & Molofsky, 2007)] calls for alternative control strategies to biological control, as the introduction of yet another alien species (control agent) might prove unsuccessful.

Food for thought

Phylogenetic relatedness is often cited as an important component of invasive potential or even the invasibility of communities (e.g. Kolar & Lodge, 2001; Strauss *et al.*, 2006*b*). The use of a 'centrifugal phylogeny' approach (Wapshere, 1974) is a common practice to assess potential nontarget effects of biological control agents whereby organisms that are most closely related to the target species are tested first, expanding to more distantly related taxa, until the full putative host range has been evaluated (Messing & Wright, 2006). Centrifugal approaches might prove particularly useful to assess the invasive potential of different taxonomic groups, the assumption being that recentness of shared ancestry between closely related species will lead to similar responses when introduced into similar environments. For example, molecular phylogenies that describe relatedness among closely related taxa that are highly invasive, moderately invasive, minimally invasive and noninvasive should reveal whether a correlation exists between genetic relatedness and invasiveness. Environmental considerations would be important, as taxa will differ in their responses in different recipient environments. Invasive taxa with differential success under similar environmental conditions are numerous, for example the Melastomataceae is one of the most devastating invasive weed families in Hawaii, with noxious taxa (e.g. *Miconia calvescens*, *Clidemia hirta*), moderately invasive taxa (e.g. *Arthrostemma ciliatum*) and less invasive taxa (e.g. *Dissotis rotundifolia*) introduced to the archipelago. Altogether 15 melastome species are currently naturalised in the Hawaiian Islands and represent an ideal system to investigate the correlation between phylogenetic distances and degrees of invasiveness. To our knowledge, such hypotheses remain untested. New and powerful risk assessment and management protocols could arise if molecular phylogenies support the current views on the phylogenetic importance in invasiveness. For example, species would be short listed as high risk if introduced to specific areas as a result of being within the identified 'phylogenetic threshold' for invasiveness for that particular group.

The nontarget impacts of introduced biological control agents are normally not easily determined (L. Kaufman, personal communication), and in many situations, such trophic interactions cannot be identified by conventional methods such as postmortem gut content analyses or real-time observations. DNA-based approaches now offer new techniques to identify gut contents of predators, providing information on the dynamics of predator–prey interactions (Hoogendoorn & Heimpel, 2001). Most research in this field focuses on the identification of one or two specific species in predator diet and has been successfully applied to most predator–prey/host systems (e.g. Agustí *et al.*, 2003; Jarman *et al.*, 2002; Juen & Traugott, 2005).

After determining native geographical sources of invaders, biological control practitioners need to differentiate specialist, host-specific enemies from generalist, less host-specific enemies, a major constraint to the timely release of control agents (M.G. Wright, personal communication). Molecular characterisation of putative biological control agent gut content in native ranges would be a fast and reliable method to distinguish generalists from specialists. Diagnostic gene amplification of species present in natural enemies' gut content would reveal diet diversity, one measure of host specificity. Laboratory simulations to evaluate host specificity are unable to recreate the vegetation structure and microclimates present in environments and could lead to changes in host preference or recognition (Symondson, 2002). However, molecular gut content characterisation would only be applicable to control agents such as Coleopteran species where the adult life stage is responsible for control and would be of little use for Lepidopteran species where the larval stage is the primary controlling stage. The recent advent and extent of 'DNA bar coding' would make species identification from molecular data relatively easy (Hebert *et al.*, 2003). Additionally, identification of gut content can also be applied to assess environmental impacts of invasive species on native biodiversity.

Border control and quarantine inspectors are not trained taxonomists and consequently many unwanted species are introduced unnoticed. Many of the examples cited throughout this review used molecular markers diagnostic for specific taxa. Training authorities in a relatively simple laboratory procedure would enhance the accuracy and rapidity of species identification and, therefore, reduce unwanted species introductions. In Hawaii, for example, all possible measures are taken to prevent the introduction of additional Melastomataceae species. Our laboratory, in collaboration with the Hawaii Department of Agriculture, is currently developing diagnostic molecular markers for particularly unwanted species that are morphologically difficult to identify, providing a basis for fast and reliable molecular identification of intercepted materials.

Conclusions

Generalised patterns in invasion ecology remain unclear and thus hamper generalisations about management and prediction of introduced species. Here, we underscored some of the recent advancements made towards effective management and control of invasive species and restoration efforts when incorporating molecular approaches. Invasion ecologists are faced with numerous challenges,

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which before molecular techniques, proved difficult and cumbersome to overcome. Ironically, these obstacles remain some of the most important aspects when management is the ultimate goal. Molecular techniques now offer new approaches to better understand the complexity of invasive organisms below and above the species level, the importance of past (e.g. dispersal), recent (e.g. hybridisation) and future (e.g. predictive) events in biological invasions and their impacts on environments. We do not intend to advocate molecular systematics to replace the tremendous amount of ongoing ecological research on invasive species but rather want to demonstrate its usefulness as a supplemental approach. Indeed, many of the inferences derived from the molecular ecology of biological invasions rely on the findings of previous ecological research. Molecular ecology as a science is still in its cradle rendering major advances and powerful inferences for future research efforts in this field.

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